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## Method development for the identification of *Russia Leather* - Comparative study of waterlogged leather samples

### Authors:

Lore G. Troalen<sup>\*1</sup>, Laurianne Robinet<sup>2</sup>, James Weatherill<sup>1,3</sup>, Sylvie Heu-Thao<sup>2</sup>, Alison N. Hulme<sup>3</sup>, C. Logan Mackay<sup>3</sup> and Elise Blouet<sup>4</sup>

### Institutions:

1- National Museums Scotland, Collections Services Department, 242 West Granton Road, Edinburgh EH5 1JA, UK

2- Centre de Recherche sur la Conservation, Musée National d'Histoire Naturelle, CNRS USR3224 CRC, Paris 75005, France

3- EaStCHEM School of Chemistry, Joseph Black Building, David Brewster Road, Edinburgh EH9 3FJ, UK

4- Atelier EBlouet, 75116 Paris, France

**City, Country:** Edinburgh, UK

**Contact:** \* l.troalen@nms.ac.uk

### Introduction

During the night of the 10<sup>th</sup> November 1786, the Catharina von Flensburg (Metta Catharina), a two-masted brigantine built 4 years before in Denmark, was sailing close to the English coast. In St Petersburg it had loaded hemp and leather and it was on its way to Genoa. As a storm was coming, the captain decided to seek shelter south of Plymouth. Unfortunately, the wind was so strong that the anchor broke and the boat crashed onto the rocks. Nothing could be saved. The men managed to reach the shore. But the boat sank taking into the depth its precious load. For nearly two centuries she lay at the bottom of the sea covered with sediments. In October 1973, while looking for another boat, underwater archaeologists found the Danish boat's bell and rolls of leather. Neither the salt nor the water had managed to damage the precious leather and soon it was exuding its tantalizing perfume of *Russia Leather* [Mouquin, 2017].

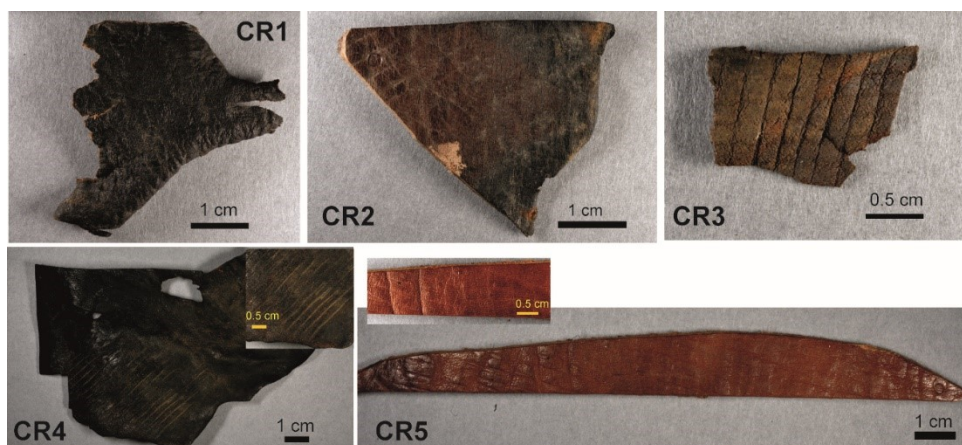
A few years later, a second and a third wreck containing *Russia Leather* were found at the bottom of the sea, one in the Netherlands, and one in Finland (Fig. 1). They both contained rolls of *Russia Leather* wrapped in hemp. And both loads were in incredible condition and dating from the end of the 18<sup>th</sup> century. When finding leather in wrecks, an immediate treatment should be applied to preserve the skins from rotting away. But, with *Russia Leather*, the hides are in perfect condition after having spent more than two hundred years in the sea. The barks that were used are the clue to this excellent state of preservation.

This leather was made in Russia. It was manufactured using bovine hides (and occasionally deer skin) and local tree bark: birch and willow. A box pattern was printed with a roll or plaque with parallel lines pressed twice perpendicularly. It was sold in brown, black or dark red colour. During the 17<sup>th</sup> and 18<sup>th</sup> century, a huge amount of *Russia Leather* was sent to Western Europe. Some years, more than a million skins were shipped out of St Petersburg. Only the best artisans were able to buy this luxury leather and create the wonderful objects seen in museums today. The impressive amount of vessels travelling through the North Sea can explain why, today, at least three wrecks containing *Russia Leather* have been found. The recipe disappeared during the Russian revolution. It was, and still is, considered as the quintessence of leather. What made it famous was its fragrance (smelling of tar, but smooth) originating from treatment of the grain with the birch oil that imparts the waterproof properties, but even more the fact that it gave the leather antifungal and insecticidal properties.

Five samples of *Russia Leather* originating from four shipwrecks were provided for analysis (Fig. 2). Their description as well as information on conservation treatment post excavation are summarized in Table 1. In order to shed more light on the materials responsible for the exceptional preservation of these leathers, an analytical approach was developed using a combination of non-invasive and micro-destructive techniques. From these techniques, information on the skin (animal species and conservation state), the presence of organic and inorganic residues, and the tannins were sought. While there are published reports on extracted tannins (sumac, oak gal) or on the analysis of tannins used in textiles [Degano et al., 2019], there are limited analytical study focusing on the analysis of tannins in historical leathers [Wouters, 1993, Falcão and Araújo, 2014, Falcão and Araújo, 2018]. This is because tannin extracts from leather are complex mixtures of small molecules, as well as cross-linked and polymeric materials, making their analysis difficult. Birch and willow barks reportedly contain condensed tannins [Falcão and Araújo, 2018] and it is reasonable to assume these will be present in leathers treated with these barks. The treatment of the grain side of leather with birch oil, may offer an additional distinguishing feature, with the presence of compounds such as salicylic acid and betulin [Boryczka, 2012]. The results obtained from the samples in this study are discussed in comparison to the available information on *Russia Leather* production.



**Fig. 1:** Recent excavation of the *Russia Leather* roll from the Juktenskobben shipwreck (© National Heritage Board Helsinki, Finland)



**Fig. 2:** *Russia leather* supplied for analysis in this research: Juktenskobben (CR1, CR2), St Nicholas (CR3), Texel (CR4) and Catharina von Flensburg (CR5).

Sample Code	Location	Date		Information on conservation treatment	State from Visual Inspection
		Wrecked	Excavated		
CR1_JUKA	Juktenskobben, Finland	N/A	2017	Rinsed with clear water	Moderate
CR2_JUKB	Juktenskobben, Finland	N/A	2017	Rinsed with clear water	Moderate
CR3_SNi	St Nicholas, Russia	1790	N/A	Not desalinated and contains iron compounds from the wreck	Poor
CR4_TEX	Texel, Netherlands	1741	2015	Rinsed with fresh water, no information on possible nourishing treatment	Moderate
CR5_CAT	Metta Catharina, Plymouth, UK	1786	1972	Rinsed with fresh water and nourished (products unknown)	Good

**Table 1:** The *Russia Leather* samples recovered from different shipwrecks around the world that were analysed in this research.

## Materials and methods

Five leather samples originating from four shipwrecks were analysed. Leather samples CR1 and CR2 both come from the Juktenskobben site in Finland, but the samples originate from two different leather rolls.

### *Infrared Spectroscopy*

Each leather fragment was analysed first by infrared spectroscopy equipped with a diamond ATR (ATR-FTIR), using a Bruker Alpha spectrometer. To avoid interference from surface deposits, these were scraped off the leather surface before analysis. The spectra were collected between 4000 and 600  $\text{cm}^{-1}$ , with 32 scans and a spectral resolution of 4  $\text{cm}^{-1}$ .

### *Differential Scanning Calorimetry*

The shrinkage temperature was measured by differential scanning calorimetry (DSC) using a Perkin DSC8000. For each leather, three samples of 1 mg were soaked into 20  $\mu\text{L}$  water for 2 hours at ambient temperature and then sealed into aluminium capsules for the analysis. Measurements were carried out between 5°C and 120°C at a speed rate of 10°C/min [Chahine, 2000], and the shrinkage temperature ( $T_s$ ) ( $T_{\text{onset}}$ ) and the enthalpy ( $\Delta H$ ) are obtained from the thermograms.

### *Water extract pH and residues*

The three 20  $\mu\text{L}$  volume of water, used to soak the leather for DSC measurements, were combined (60  $\mu\text{L}$ ) and the pH of the water extract measured using a pH meter equipped with a micro electrode. Subsequently, the water extract was dried out on a glass slide at ambient temperature and the solid residue analysed by ATR-FTIR.

### *Proteomic analysis*

For the identification of the animal species by proteomic analysis, a few leather fibers were sampled (~10  $\mu\text{g}$ ) and analysed according to the peptide mass fingerprinting method [Kirby 2013]. Collagen samples were fragmented into peptides by trypsin digest. After purification and concentration, the digested samples were analysed using a MALDI-TOF/TOF (AB SCIEX) mass spectrometer equipped with a Nd:YLF laser at 345 nm in positive mode and reflectron

mode (700-4000 Da range). The accuracy on the detected mass is 50 ppm. The animal species was identified after comparison of the obtained spectra with a reference database.

#### *X-ray Fluorescence analysis (XRF)*

XRF was carried out with an Oxford Instruments ED 2000 air-path with Rh target X-ray tube collimated to a point of approx.  $4 \times 3.5$  mm<sup>2</sup>, coupled to a Si(Li) detector. Spectra were collected under “Old XRF” conditions (46 kV, 1000  $\mu$ A, 45% dead time, no primary beam filter) and the analysis was qualitative. The spectral deconvolution was performed using ED 2000SW software. The detection limit varies depending on the elements, matrix and analytical conditions, but is typically in the range of 0.05% – 0.2%.

#### *Liquid Chromatography and Mass Spectrometry*

##### Extraction

The extraction protocol was optimized based on the early work on historical leather [Wouters 1993]. 1 mg of leather sample was soaked for 48 hours in 200  $\mu$ L of Acetone: H<sub>2</sub>O (1:1, v/v) and then sonicated for 1 hour. The extract was then centrifuged at 8000 rpm for 30 min and 100  $\mu$ L of the supernatant was collected. After drying the extracts were reconstituted in 50  $\mu$ L H<sub>2</sub>O:MeOH 0.05% HCOOH (3:1, v/v) and 10  $\mu$ L injected for analysis.

##### Ultra-High Performance Liquid Chromatography (UHPLC-PDA)

The UHPLC chromatographic method was developed using a Waters Acquity UPLC™ I-class system with sample detection using a Waters PDA detector (210 to 800 nm). Data was collected by Waters Empower 2 software. Sample extracts were automatically injected *via* a Rheodyne injector with a 10  $\mu$ L sample loop. The bandwidth (resolution) was 1.2 nm with a sampling rate of 5 points s<sup>-1</sup>. The method used a C18 reverse phase column, 1.7  $\mu$ m particle size, 50  $\times$  2.1 mm (length  $\times$  i.d.), set-up with an inline filter. The total run time was 10.33 min at a flow rate of 700  $\mu$ L min<sup>-1</sup> and the column was maintained at  $55 \pm 1$  °C. A binary solvent system was used; A = 0.05 % aqueous HCOOH (pH 3), B = MeOH with 0.05 % HCOOH. The elution program was isocratic for 1 min (95A: 5B), a linear gradient from 1 min to 7.5 min (30A: 70B), then a linear gradient from 7.5 min to 8 min (90A: 10B) before recovery of the initial conditions over 0.1 min and equilibration over 1.9 min [Weatherill et al., In press]. Several pure chemical standards including 3,4-dihydroxybenzoic acid, catechin, and ellagic acid were used to build-up a database of tannins.

##### Accurate Mass data (FTMS)

The CR5 extract was investigated using ultra high resolution mass spectrometry, acquired on a 12T Solarix XR FT-ICR (Bruker Daltonics). Samples were introduced into the mass spectrometer by laser desorption ionization (LDI) using a smartbeam 2 laser operating at 2 kHz. Spectra were acquired in negative mode and were the results of 20 summed acquisitions measured at 2 Mword with a mass range of  $m/z$  150-3000. The resulting mass spectra had an average peak resolution of 200,000 and a typical mass accuracy of less than 1 ppm, facilitating the determination of a unique molecular formula based on the observed accurate mass. Data analysis was performed using Data Analysis version 5.0 (Bruker Daltonics).

## **Experimental Results**

### *Global analyses*

Visually, all the leathers display the box pattern on the grain side characteristic of Russian leathers (Fig 2). The samples were degraded to varying degrees, mostly with a loss of flexibility and an increase rigidity. Among those, the *Metta Catharina* sample (CR5) appeared the best preserved retaining the flexibility of a new sample.

The infrared analyses of the leather give an insight into the main constituents and their preservation (Fig 3A). With the exception of CR3, all the spectra display absorption bands around 2928 & 2856 cm<sup>-1</sup> associated with the presence of an oil residue, as well as strong

amide I & II peaks around 1630 and 1540  $\text{cm}^{-1}$  respectively, indicative of the preserved protein, in this case collagen. The infrared spectrum of CR3 shows very weak protein peaks and a strong signal associated with the presence of an inorganic compound, possibly an iron oxide considering the higher iron content detected by XRF in this sample (Fig. 4). In addition, XRF analysis showed the presence Ca, K, Cu, Zn and Sr in all of the samples, while Pb, Br and Mn were detected in some samples.

The pH of the water extract for all leathers is high, with values ranging from 5 to 6.7 (Table 2). In a vegetable tanned leather, the expected pH is generally around 4 to 4.5, therefore the increase likely result from the exposure to sea water (which generally has a pH around 8).

#### *Collagen analysis*

From proteomic analysis, all of the leathers were found to be made from bovine skin. The measurements of the shrinkage temperature and the enthalpy by DSC (Fig 5, Table 2) confirm the trends observed in FTIR spectra. In leathers CR1, CR2 and CR5 the collagen is well preserved with  $T_s$  between 72 and 84°C and high  $\Delta H$  (13-22 J/g). In contrast, very little collagen is retained in leather CR3 and CR4 considering the very low  $\Delta H$  (2-3 J/g). The poor state of preservation of CR3 and CR4 might be explained by the high quantities of iron detected by XRF (Fig. 5), as it is well documented that the presence of Fe promotes the rate of degradation of natural textile fibres, including proteinaceous fibres, such as silk and wool [Wilson et al., 2011].

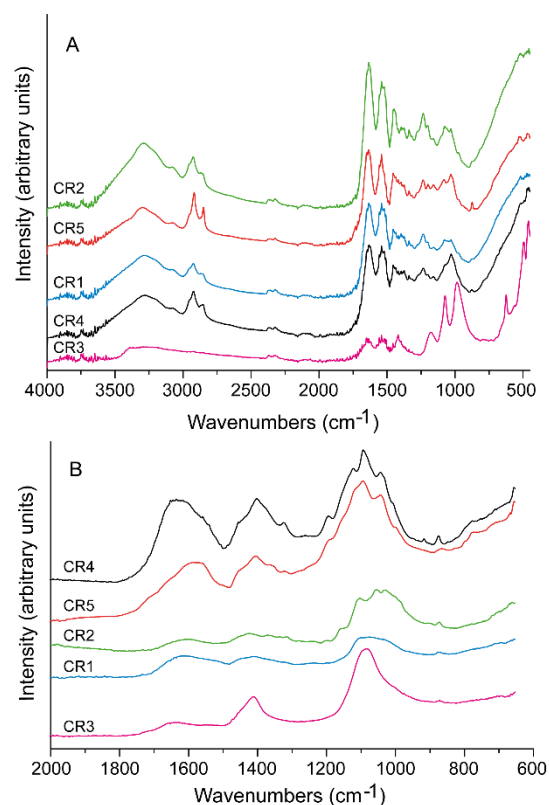
#### *Tannins and dyes analysis*

FTIR analyses of the dried residues from the aqueous extracts, allowed the analysis of water soluble compounds such as tannins in the absence of collagen signals which would otherwise dominate the spectra (Fig. 3B). The extracts from CR4 and CR5 display a similar spectral profile which is characteristic of a vegetable tannin [Falcão and Araújo, 2014]. The signal intensity, the peak position and peak resolution in the spectra of leathers CR1, CR2 and CR3, suggest the compounds extracted are different and/or more degraded. From FTIR spectra, discrimination between hydrolysable and condensed tannins could not be achieved in this case [Falcão and Araújo, 2014], hence more in depth analyses using chromatographic techniques were undertaken.

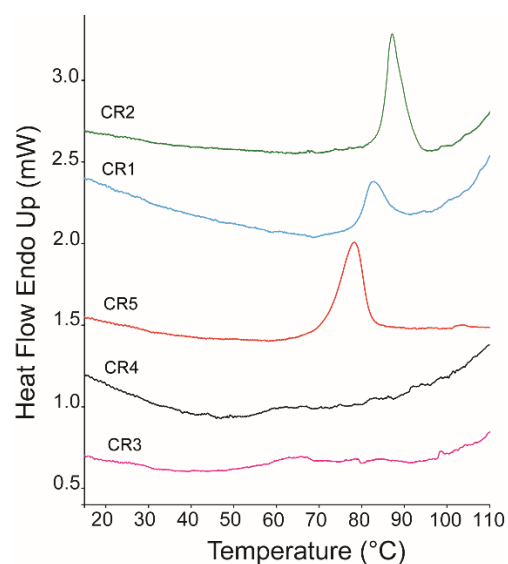
The chromatographic profiles obtained by UHPLC from the leather extracts (at 254 nm) are displayed in Fig. 6 and Table 3 summarizes the chromophores characterized. From this analysis, it seems that the 5 samples of *Russia Leather* contain residual tannins and display some similarities. Identified in the extracts are 3,4-dihydroxybenzoic acid and a related compound, as well as minor levels of ellagic acid. 3,4-Dihydroxybenzoic acid is a degradation product of catechin, a condensed tannin and both these compounds have been characterized in birch and willow bark extracts [Weatherill et al., *in press*]. The relative quantity of 3,4-dihydroxybenzoic acid was found to be higher in CR5 samples, while CR1 and CR3 samples contain a lower level of preserved tannins. In several samples urolithin C, which is a well-known degradation compounds of the redwood dyes [Peggie et al., 2018] was detected, probably used to colour the leather. Traces of yellow chromophores were also observed in several samples but could not always be attributed. Residual sulfuretin was detected in CR5, which correspond to a yellow dye present in the young fustic wood (*Cotinus Coggygria* Scop.).

In order to confirm these attributions and to explore the potential of an advanced technique used for complex mixture analysis [Kew et al., 2018], the extract from CR5 was investigated by Mass Spectrometry accurate mass analysis (FTMS). The mass spectrum obtained (Fig. 7) is typical of a complex mixture. Several compounds were isolated according to their chemical formula. This confirmed the presence of the chromophores identified by UHPLC analysis (3,4-dihydroxybenzoic acid, ellagic acid, urolithin C), but also allowed the detection of additional compounds including the flavonol myricetin and the flavonol morin or quercetin (Table 4). The analysis of the birch and willow bark extracts is currently on-going, however from preliminary

analysis, willow bark extract also contains the flavonols myricetin and quercetin [Weatherill et al., *in press*]. Finally, Betulin, one characteristic compound from birch oil, was not detected in the CR5 extract.



**Fig. 3:** (A) FTIR-ATR spectra of the *Russia Leather* samples (grain side); (B) FTIR spectra of the tannins extracted from the *Russia Leather* samples.

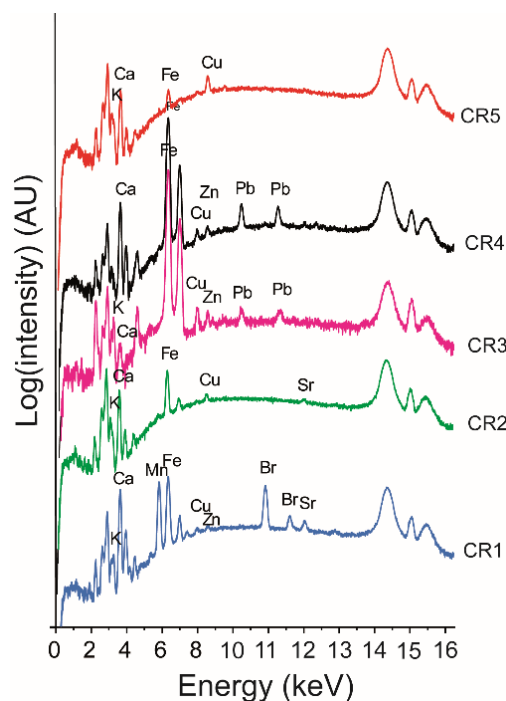


**Fig. 4:** DSC thermograms from the *Russia leather* samples.

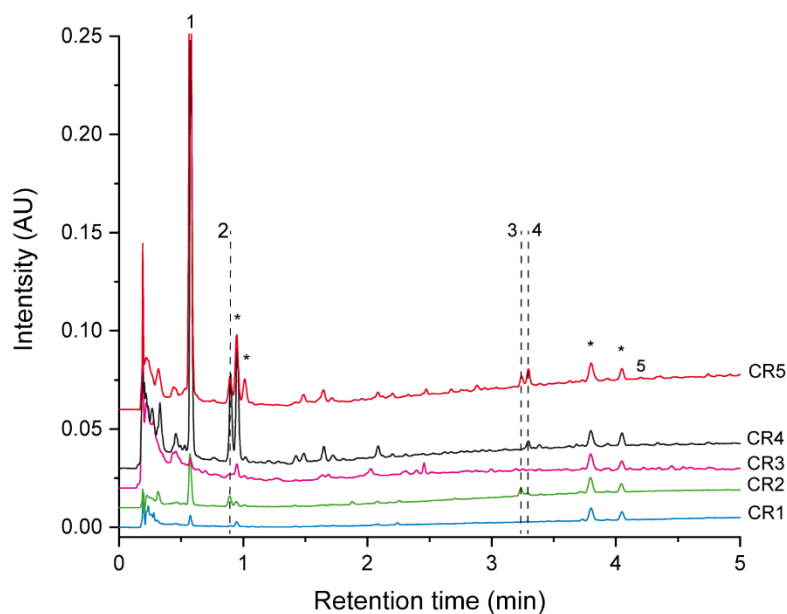
**Table 2:** Values of the pH, shrinkage temperature ( $T_s$ ) and enthalpy ( $\Delta H$ ) measured for the different Russia leather samples.  $T_s$  and  $\Delta H$  values were averaged over 3 measurements.



	pH	Td (°C)	$\Delta H$ (J/g)
CR1	6.69 $\pm$ 0.04	78.8 $\pm$ 0.8	13.1 $\pm$ 5.2
CR2	6.60 $\pm$ 0.02	83.7 $\pm$ 1.4	21.9 $\pm$ 2.6
CR3	5.11 $\pm$ 0.11	53.0 $\pm$ 1.6	3.4 $\pm$ 0.8
CR4	5.67 $\pm$ 0.12	56.1 $\pm$ 1.2	2.6 $\pm$ 1.3
CR5	5.48 $\pm$ 0.02	71.9 $\pm$ 2.7	18.1 $\pm$ 5.8



**Fig. 5:** XRF analysis of the samples displayed in Log10 for the intensity. Note the high quantities of iron detected in CR3 and CR4 samples.

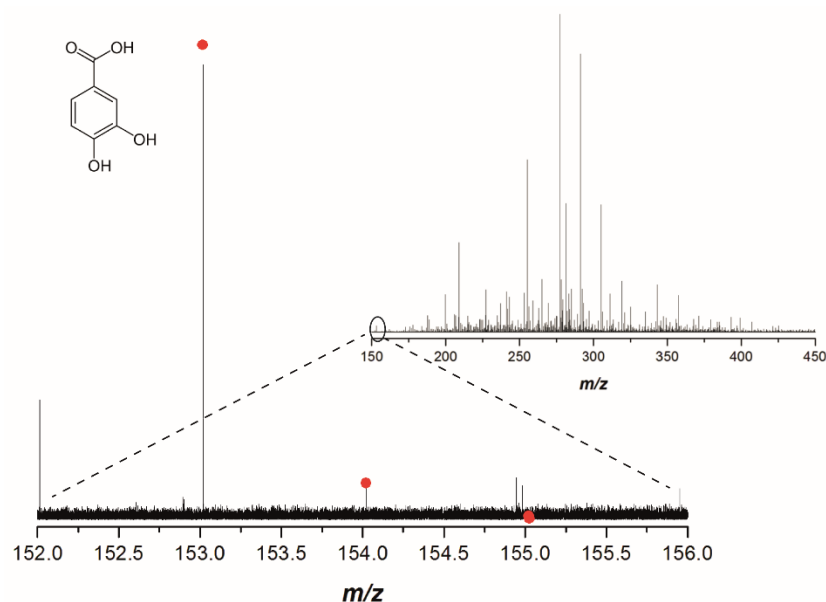


**Fig. 6:** Comparison of the extracts of sample CR1-5, monitored at 254 nm. Identified in the chromatograms are (1) 3,4-dihydroxybenzoic acid, (2) a compound related to 3,4-dihydroxybenzoic acid; (3) ellagic acid, (4) urolithin C; (5) sulfuretin. Compounds marked \* all display a  $\lambda_{\text{max}}$  absorption at 254-255 nm, but with no particular features.



Entry	CR1	CR2	CR3	CR4	CR5
3,4-dihydroxybenzoic acid (0.53 min)	x	x	x	x	X
Related to 3,4-dihydroxybenzoic acid (0.89 min)		x	x	x	x
Ellagic acid (3.15 min)		x		x	x
Urolithin C (3.25 min)		x		x	x
Sulfuretin (4.20 min)					x

**Table 3:** Compounds identified in CR1-5 samples based on UHPLC-PDA analysis.



**Fig. 7:** FTMS analysis of CR5 with isolation of 3,4-dihydroxybenzoic acid or 3,5-dihydroxybenzoic acid ( $C_7H_6O_4$ ). Red dots illustrate the expected isotopic distribution.

Entry	Formula	Observed	Predicted	Error (ppm)
3,4-dihydroxybenzoic acid or 3,5-dihydroxybenzoic acid	$C_7H_6O_4$	153.01794	153.01933	-9.08
Ellagic Acid	$C_{14}H_6O_8$	300.99875	300.99899	-0.80
Myricetin	$C_{15}H_{10}O_8$	317.02997	317.03029	-1.01
Urolithin C	$C_{13}H_8O_5$	243.03024	243.0299	1.40
Quercetin or Morin	$C_{15}H_{10}O_7$	301.03514	301.03538	-0.80

**Table 4:** Compounds identified in CR5 sample by FTMS analysis (negative mode).

## Discussion and further work

This first analytical study of a corpus of archaeological *Russia Leathers* was an opportunity to gain information on the constitutive materials of this special leather, to assess their preservation after over two centuries in sea water and to develop an analytical methodology for characterization.

Visually the leather samples appeared to be well conserved and overall the chemical analyses conducted confirmed this, with strong evidence for the preservation of the main constituents the collagen, the tannin and the dyes. In most of the leathers it was possible to confirm the use of a bovine skin, the likely tanning with birch and willow bark from the identification of degraded catechin and the presence of redwood dyes. However, the use of birch oil could not be confirmed at this stage.

Differing states of conservation are observed between the leathers, even within the same excavation site, likely due to their exposure to different environments. The leather CR3 from the St Nicholas shipwreck was the most degraded, likely due to the presence of high quantities of iron. The sample was highly mineralised with almost no collagen retained, nevertheless, a small amount of degraded catechin could still be detected. The extensive degradation probably results from the fact that no conservation treatment was applied to the leather following excavation, in particular the absence of desalination after the exposure to iron compounds. Although the two leathers CR1 and CR2 both came from the site of Juktenskobben in Finland, they displayed different states of conservation, and this was observed for all the analyses, which showed a higher degradation of the CR1 sample. Finally, the leather coming from the Metta Catharina (CR5) was the best from the chemical analyses point of view, confirming the visual observation.

The analytical methodology proved well suited to characterize a complex material such as *Russia Leather* and will be transferable to the analysis of other vegetable tanned leather. The analysis of tannins still remains a challenge due to the presence of complex mixtures of small molecules, for that reason future work will focus in that area. This first application of FTMS analysis to investigate tannins gave very promising results, therefore the technique will be applied to the other *Russia Leather* samples to confirm the compounds present in their extracts and compare them with the extracts from birch and willow barks. Different sample preparation will be also explored in order to gain information from the original lubricants.

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### Contributions

Conceptualization, LT, LR, EB; methodology, LT, LR; formal analysis, LT, LR, SHT, JW, CLM; data treatment, LT, LR, JW, SHT, ANH, CLM; writing—original draft preparation, LT, LR, EB; writing—review and editing, LT, LR, JW, SHT, ANH, CLM, EB; funding, LT, LR

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